Two clone system to determine complex signaling between wild-type and Aβ42 expressing neurons in Alzheimer's Disease

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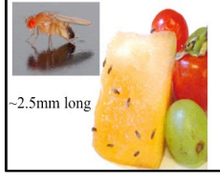
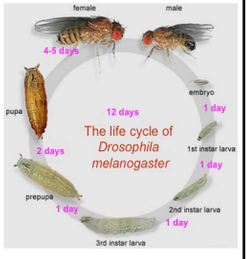
Abstract

Alzheimer's Disease (hereafter AD) is an irreversible neurodegenerative disease causing death of millions of elderly people every year. One of the reasons for AD is abnormal cleavage of the Amyloid precursor protein (APP), which forms a 42 amino acid long hydrophobic polypeptide (hereafter Aβ42) which form aggregates leading to amyloid plaques. This Aβ42 forms plaque that leads to neuronal cell death. We are trying to understand the genetic underpinnings behind the onset of this deadly disease using Drosophila melanogaster eye as our model system. We have generated and optimized a two-clone system in our lab to understand the crosstalk between the plaque forming neurons and the adjacent wild type neurons. The onset of AD initiates with a few neurons which start producing amyloid plaques, which then triggers cell death. One of the questions is: Which neuronal population is affected? Our system utilizes the FLP/FRT mediated recombination to produce two types of neuronal cell population where strong GFP reporter marks the Aβ42 misexpressing neurons, and the neighboring wild-type neurons are marked by the absence of GFP. Our preliminary data suggests that the Aβ42 misexpressing neurons survive at the expense of the neighboring wild type cells. Thus, we believe that there are certain signals, which emanates from these Aβ42 plaque producing neurons towards the wild type neurons, which causes them to die. We have identified evolutionarily conserved Jun-N-Terminal Kinase (JNK) Signaling pathway as one of the genetic modifiers of Aβ42 mediated neurodegeneration, which induces neuronal death. With the help of our two-clone system, we want to understand which neuronal cells (Aβ42 misexpressed vs wild type cells) and how, the JNK signaling triggers cell death. We will test reporters and antibodies against the members of JNK signaling pathway to address our hypothesis. Furthermore, identifying the genetic biomarkers of the Alzheimer's disease with the help of our genetic tool can be utilized in finding therapeutic targets in the future.

Drosophila melanogaster *a.k.a* fruit fly is Cindrella of modern genetics

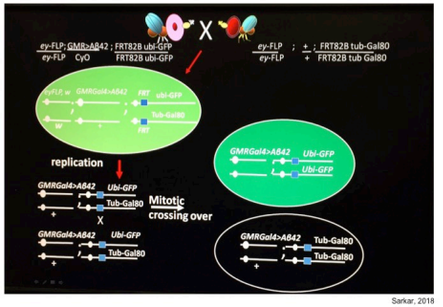
Kingdom:	*Animalia*
Phylum:	*Arthropoda*
Class :	*Insecta*
Order :	*Diptera*
Family:	*Drosophilidae*
Genus:	*Drosophila*
Species:	*melanogaster*



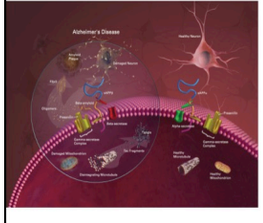


Two Clone System

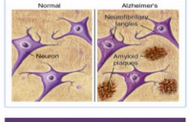
To study crosstalk between Aβ42 producing cells and wild-type cells

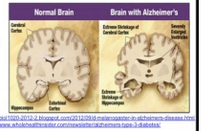


Alzheimer's Disease



1. Amyloid Plaques
2. Neurofibrillary Tangles
3. Oxidative stress due to ROS
4. Neuronal loss
5. Genetic basis of ApoE.

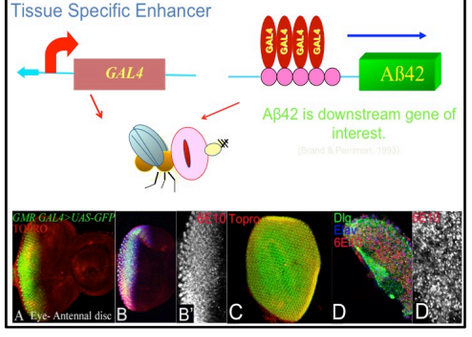




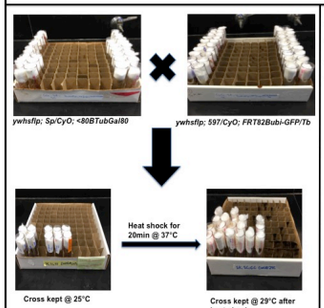
- Progressive neurodegenerative disorder
- Caused by miscleavage of a transmembrane APP protein
- Sixth largest killer
- 1 in 3 individual above the age 65 may manifest Alzheimer's Disease.
- No cure to date

http://dart1503-2012-2.blogspot.com/2012/09/d-melanogaster-in-alzheimers-disease.html
http://www.advancedhomecare.com/newsletter/alzheimers-type-3-diabetes/

Gain of-function approach: GAL4/UAS- System



Materials and Methods



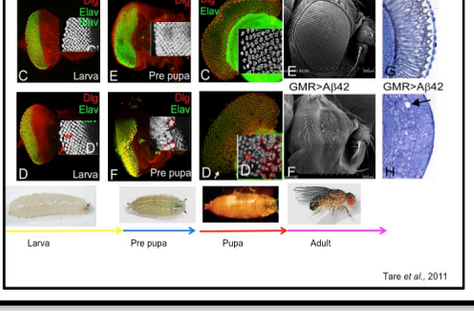
- 3rd Instar Larvae from 29°C were dissected
- Samples were washed in Primary and Secondary Antibody
- Eye Discs were separated and mounted
- Mounted samples were imaged using Confocal Microscope

Adult Flies can be used for adult eye imaging

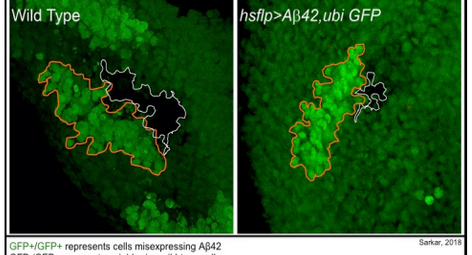
The power of *Drosophila* model


- Genome sequenced (~180Mb), 4 chromosomes, 15000 genes.
- Most genes are conserved between flies and humans. Less genetic redundancy.
- Basic cell biological pathways are nearly identical in flies and humans (eye specification, cell cycle, Ras, p53, InR signaling...)
- Tissue-specific knock-outs (clones of mutant cells)
- Ideal human disease model for genome wide genetic screens

Misexpression of Aβ42 leads to progressive neuronal cell death



Results and Future Directions



GFP+/GFP+ represents cells misexpressing Aβ42
GFP-/GFP- represents neighboring wild-type cells
Cells misexpressing Aβ42 are growing at the expense of the neighboring wild-type cells

Future Directions

The next step for this project is to test Wingless and JNK pathways to further determine complex signaling between GFP+/GFP+ and GFP-/GFP- cells and understand why neuronal loss is occurring at unequal rates

We would like to thank Bloomington Stock Center & DSHB for reagents. AS is supported by NIH 1R15GM124654-01 and Stem Catalyst Grant. AS is supported by Department of Biology, University of Dayton. AS is supported by Office for Graduate Academic Affairs through Graduate Student Summer Fellowship Program (GSSF) and Department of Biology, University of Dayton.